Association of longer QT interval with arterial waveform and lower pulse pressure amplification: the Nagahama Study.

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TITLE:

Association of serum free fatty acid level with reduced reflection pressure wave magnitude and central blood pressure: The Nagahama Study

SHORT TITLE:

Free fatty acid and central hemodynamics

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ABSTRACT

Central blood pressure (BP) has been suggested to be a better predictor of cardiovascular disease risk than brachial BP. Given that central BP and arterial waveform are both influenced by insulin resistance, major initiators of insulin resistance—such as serum free fatty acid (FFA)—are suspected of potentially being involved in central hemodynamics. To confirm that insulin signaling is an important modulator of central hemodynamics, we investigated this hypothesis in a large-scale general population. Brachial BP and radial arterial waveform were measured simultaneously in 9,393 middle-aged to elderly individuals. The augmentation index (AIx) was calculated from the radial waveform as the ratio of the height of the late systolic peak to that of the first peak. Central systolic BP was defined as the absolute pressure of the late systolic peak of the waveform. Differences in central and brachial pulse pressure (PP) were considered to represent PP amplification. PP amplification differed significantly among serum FFA level quartiles (Q1, 7.8±5.3; Q2, 8.6±5.0; Q3, 9.3±5.7; Q4, 10.3±6.1 mmHg, $P<0.001$), and the maximum difference in combination with diabetes status was 4.9 mmHg. Multivariate analysis adjusted for major covariates indicated that higher serum FFA was an independent determinant for higher PP amplification ($\beta=0.145$, $P<0.001$) and lower AIx ($\beta=-0.122$, $P<0.001$) and central systolic BP ($\beta=-0.044$, $P<0.001$), while the association between FFA and PP amplification significantly decreased ($\beta=0.022$, $P<0.001$) after further adjustment for AIx. Serum FFA is an overlooked factor favorably influencing central hemodynamics. A low-magnitude reflection pressure wave might be involved in this paradoxical relationship.

KEYWORDS:
Central hemodynamics, arterial waveform, insulin resistance, free fatty acid

INTRODUCTION

Hypertension is a leading cause of cardiovascular disease, with brachial blood pressure (BP) being a standard measure in the assessment of arterial pressure load. However, central BP estimated from the radial arterial waveform has recently been suggested to be more closely associated with cardiovascular outcomes than brachial BP [1-3]. In addition to these epidemiological findings, clinical studies from several groups [4-6] and our own [7] have suggested that antihypertensive drugs might exert different effects on arterial waveform and central BP, possibly resulting in different cardiovascular outcomes. The Conduit Artery Function Evaluation sub-study [4] of the Anglo-Scandinavian Cardiac Outcomes Trial demonstrated that calcium-channel blockers were superior to β-blockers for reducing cardiovascular events. This effect was presumably due to the central systolic BP (SBP) being lower in the calcium-channel blocker treatment arm, while no class-specific effects were observed regarding brachial SBP. The apparent influence of central BP on cardiac outcomes highlights the importance of identifying factors that might affect central BP levels.

Several factors have been reported to influence central BP levels by altering the arterial pressure waveform [8], a composite waveform of the forward pressure wave generated by cardiac ejection and the backward pressure wave reflected at peripheral sites. Arterial stiffness causes the early return of reflection pressure waves from peripheral sites and thus increases overlaps between forward and reflection pressure waves at the aorta, which increase central SBP. Other factors also influence arterial waveform, such as tall stature greatly decreasing the overlap of the two waveforms by delaying the arrival of the reflection pressure wave, and increased heart rate (HR) reducing the overlap by shortening the cardiac ejection period.

Curiously, type 2 diabetes and insulin resistance have been favorably associated with central hemodynamics. Several groups [9, 10] and our own [11] have shown that individuals with type 2 diabetes had relatively low central SBP, despite the well-established pathogenicity of diabetes for arterial stiffness and cardiovascular diseases. Although the mechanisms behind this paradoxical relationship are unclear, a possible explanation is reduced magnitude of the reflection pressure wave [12] due to a stiffer aortic artery and consequently larger penetration of pulsatile energy into the microcirculation [13, 14]. Insulin-mediated vasoconstriction under insulin-resistant conditions [15] might also be involved in the increased transmission of pulsatile energy.

Free fatty acid (FFA) is a major initiator of insulin resistance [15, 16]. FFA blocks insulin signaling via phosphorylation of insulin receptor substrate 1, which inhibits translocation of glucose transporter to the cell membrane and reduces glucose uptake [15]. Further, FFA has been shown to reduce endothelium-dependent vasodilation by decreasing endothelial nitric oxide (NO) production [14]. Given these molecular bases of FFA in initiation of insulin resistance, we hypothesized that serum FFA levels might also be associated with central hemodynamics. Proving our hypothesis would further support the involvement of insulin signaling in central hemodynamic control, and would help to further understand the basis of paradoxical relationship between insulin resistance and better central hemodynamic profile.

Here, we investigated our hypothesis using a dataset from the Nagahama Prospective Cohort for Comprehensive Human Bioscience (the Nagahama Study), a large-scale population-based cohort study in Japan.

METHODS

Study subjects

Study subjects were 9,393 apparently healthy middle-aged to elderly citizens who had participated in the Nagahama Study. This study cohort was recruited from 2008 to 2010 from the general population of Nagahama City, a largely sub-urban city of 125,000 inhabitants in central Japan. Community residents aged 30 to 74 years, living independently and with no physical impairment or dysfunction, were recruited. Of 9,804 total subjects, those meeting any of the following conditions were excluded from this study: history of symptomatic cardiovascular diseases (n=266), taking insulin therapy (n=22), unsuccessful assessment of clinical parameters required for this study (n=80), and pregnant women (n=43).

Of the 9,393 subjects remaining after exclusion, individuals with available fasting blood specimens (>11 h) were used as the study panel (n=4,322), while those with peripheral blood samples drawn within 10 h of their last meal were used as the replication panel (n=5,071).

All study procedures were approved by the ethics committee of Kyoto University Graduate School of Medicine and the Nagahama Municipal Review Board. Written informed consent was obtained from all participants.

BP measurement

Radial arterial waveform, brachial BP, and HR were measured simultaneously (HEM-9000AI; Omron Healthcare, Kyoto, Japan) after 5 min resting in the sitting position. Briefly, brachial BP was measured at the right upper arm using a cuff-oscillometric device, and the radial arterial waveform was simultaneously obtained from the left wrist using a multi-element tonometry sensor. The augmentation index (AIx) was calculated from the radial

arterial waveform as the ratio of the height of the late systolic peak (SBP2) to the first systolic peak. The absolute pressure of SBP2 obtained by calibrating the first systolic peak with brachial SBP was considered to represent the central SBP. Pulse pressure (PP) amplification was calculated by subtracting central PP from brachial PP and expressed in absolute values (mmHg). Measurements were taken twice, and the mean value of these measurements was used in analysis. The validity of SBP2 in estimating central SBP has been demonstrated by invasive simultaneous measurement of the ascending aorta and radial artery pressure [17, 18]. We also previously reported that radial SBP2 was closely related to the central SBP calculated by the widely used generalized transfer function [19]. Mean BP (MBP) was calculated using the following formula: MBP=DBP + (SBP − DBP)/3.

Clinical parameters

Basic clinical parameters were measured at the baseline examination of the Nagahama cohort study. Serum FFA levels were quantified using an enzymatic assay (NEFA-HR; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Intra- and inter-assay coefficients of variation in FFA measurements were 1.42% and 1.79%, respectively. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as an index of insulin resistance using the following formula: [insulin (IU/l) × glucose (mg/dl)]/405.

Assessment of arterial stiffness

Arterial stiffness was assessed by pulse wave velocity (PWV) measured between the brachia and ankle (baPWV). Briefly, cuffs were applied to both brachia and ankles, and blood pressure was measured simultaneously in the supine position using a cuff-oscillometric device

(Vasera-1500; Fukuda Denshi, Tokyo, Japan). Pulse volume waveforms were also recorded simultaneously using a plethysmographic sensor connected to the cuffs. The baPWV was calculated from the time interval between the wave fronts of the brachial and ankle waveforms and the path length from the brachia to ankle (0.597 × height + 14.4014) [20]. The co-linearity of baPWV with a carotid-to-femoral PWV, a standard measure of arterial stiffness, has been previously reported [21].

Statistical analysis

Quartile of PP amplification and serum FFA level was calculated for each sex and then combined to avoid potential sex differences. Differences in numeric parameters among subgroups were assessed by analysis of variance, while the frequency of differences among subgroups was evaluated using a chi-squared test. Factors independently associated with PP amplification and AIx were assessed by multiple linear regression analysis. Statistical analysis was performed using JMP 9.0.3 software (SAS Institute, Cary, NC, USA). A p-value of less than 0.05 indicated statistical significance.

RESULTS

Clinical characteristics of study subjects are summarized in Table 1. Plasma levels of triglycerides, insulin, and FFA were slightly higher in the replication panel than in the study panel (P<0.001), while no marked differences were observed for other parameters.

Table 2 shows the differences in metabolic parameters among quartiles of PP amplification. Subjects with larger PP amplification were markedly younger and taller and had faster HR than those with less amplification. Although several clinical parameters

significantly differed among quartiles in crude analysis, parameters for insulin resistance, including FFA levels, remained significant even after adjustment for major covariates.

As a whole, women had significantly higher FFA levels than men (Figure 1). Older age (r=0.087, P<0.001), lower BMI (r=-0.084, P<0.001), increased HDL (r=0.190, P<0.001) and total cholesterol levels (r=0.085, P<0.001), and higher brachial SBP (r=0.057, P<0.001) had a significant but weak association with serum FFA levels (Tables S1 and S2). PP amplification markedly increased with FFA quartile (Figure 2), although both brachial and central SBP also showed linear association with FFA levels (Figure S1). In combined analysis with diabetes status (Figure 2C), differences in PP amplification between the highest (diabetic individuals with highest FFA quartile) and lowest (non-diabetic controls with lowest FFA quartile) subgroups reached approximately 4.9 mmHg. In contrast, AIx exhibited an inverse association with FFA quartile (Figure 2D), while baPWV was positively associated with FFA quartile (Figure 2E).

Table 3 summarizes the results of multiple regression analysis for central hemodynamic parameters. Results indicated that serum FFA level was an independent positive determinant for PP amplification (Model 1). Given that FFA was also strongly and inversely associated with AIx, we further adjusted AIx in the regression analysis (Model 2). Although the association between serum FFA and PP amplification remained significant, the regression coefficient of FFA substantially decreased. Lower AIx might therefore be involved in the relationship between elevated serum FFA levels and elevated PP amplification. Further, FFA overtook the positive association between plasma insulin level and AIx (Models 3 and 4). Results of these regression analyses indicated that serum FFA levels rather than plasma insulin concentration is a key factor in reducing AIx in subjects with insulin resistance. Waist

circumference was not identified as an independent determinant when included instead of body weight in regression Model 4 (P=0.466). The association of FFA with AIx might be independent of adiposity. Serum FFA level also showed an inverse and independent association with central SBP after adjustment for brachial SBP (Model 5).

FFA was a positive determinant for arterial tone when assessed via baPWV (Model 6). However, the associations of FFA with AIx (Model 4), as well as central pressure (Model 1, 2, and 5), were independent of baPWV, suggesting that changes in reflection magnitude rather than transit time of the reflection pressure wave might be involved in the paradoxical relationship between higher FFA and better central hemodynamic profiles.

These findings were supported in the analysis using the replication panel irrespective of potential differences in fasting status, and no marked sex differences were found in any regression model (Table S3). When MBP was adjusted in the regression models instead of SBP, no marked changes were observed in the regression coefficients of FFA, as follows: Model 2 (PP amplification), β=0.030, P<0.001; Model 4 (AIx), β=-0.115, P<0.001; Model 5 (cSBP), β=-0.016, P<0.001; and Model 6 (baPWV), β=0.073, P<0.001. Further, the association of FFA with central hemodynamic parameter was independent of glycemic control levels assessed by HbA1c: HbA1c-adjusted regression coefficients of FFA; Model 2, β=0.022, P<0.001; Model 4, β=-0.123, P<0.001; Model 5, β=-0.044, P<0.001; and Model 6, β=0.069, P<0.001.

DISCUSSION

In the present study, we clarified that elevated serum FFA levels were strongly associated with increased PP amplification and decreased AIx, which represents relatively low central

BP, in a large-scale general population sample. To our knowledge, this is the first report of a favorable association of FFA with central BP and arterial waveform, which suggests the importance of insulin signaling as a modulator of central hemodynamics. Reduced magnitude of the reflection pressure wave might be involved in this paradoxical relationship.

Insulin resistance and diabetic status have been shown to be favorably associated with AIx and central BP in observational studies in patients with diabetes [22, 23] and general populations [9, 13], as well as in an experimental study using a euglycemic insulin clamp technique [24]. We also reported that not only increased insulin resistance but also reduced insulin sensitivity assessed by an oral glucose tolerance test were factors that modulate the arterial waveform and reduce central BP [12]. In the present study, however, serum FFA was a more prominent determinant of AIx than insulin. FFA initiates insulin resistance via upstream inhibition of insulin signaling in target cells, while increased plasma insulin levels or hyperinsulinemia are secondary responses to compensate for reduced glucose uptake under conditions of insulin resistance. The phase difference in the roles of FFA and insulin may explain the stronger association of FFA with AIx.

In stiffer arteries, the stiffness gradient from aorta to resistant artery was progressively dissipated. Decreased stiffness gradient reduces partial reflection of the forward pressure wave and increases transmission of the pulsatile energy into peripheral microcirculation, which reduces the magnitude of reflection [14]. Chirinos et al. recently observed selective stiffening of the aorta, but not more distal arteries, in patients with type 2 diabetes and suggested that this selective stiffening was the underlying mechanism for the paradoxical observation of a lower reflection magnitude in subjects with type 2 diabetes [13]. Odaira et al. also reported that the contribution of the wave reflection to central

hemodynamics might be reduced in subjects with relatively stiff arteries [25]. As baPWV and AIx was inversely associated with FFA quartile, i.e. faster baPWV and lower AIx in higher FFA quartiles, our findings support the “pulsatile energy” hypothesis.

FFA plays a key role in the initiation of insulin resistance by inhibiting glucose uptake of target cells [15]. Given the importance of endothelium-derived NO in vascular relaxation shown in an animal model of hypertension [26], the decreased NO production at the endothelium and subsequent endothelial dysfunction are concomitant mechanisms for the development of insulin resistance via FFA [15]. As our study participants were an apparently healthy general population without severe insulin resistance, reduced endothelial NO production might be a principal factor in the increased aortic tone and consequently larger pulsatile energy in subjects with higher FFA. Insulin increases aortic tone by activating the sympathetic nervous system under the condition of insulin resistance. However, given the weak relationship between serum FFA and insulin levels, the involvement of insulin-mediated sympathetic activation might be independent of the effect of FFA. This is supported by the results of our regression analysis that show the insulin-independent association of FFA with baPWV.

We also investigated associations between plasma lipid parameters and PP amplification, but no remarkable relationships were observed after adjustment for basic covariates. These results further emphasize the importance of serum FFA, but not lipid profile, as a factor involved in central hemodynamics. A previous study in Australia [27] reported that obesity, particularly visceral adiposity, was significantly associated with smaller AIx. As serum FFA is mostly released from enlarged and stressed adipose tissue [28], FFA might be a confounding factor in the inverse association between visceral adiposity and smaller AIx. No

association between waist circumference and AIx was observed in the present study, which supports our hypothesis.

The maximum difference in PP amplification among all FFA quartiles was approximately 2.5 mmHg. This BP difference was somewhat larger than that observed in our previous reports of the association of smoking intensity [29] and insulin sensitivity [12]. The combination of FFA quartile and type 2 diabetes status further increased the maximum PP difference to 4.9 mmHg. A previous clinical study, the CAFE study [8], clearly indicated that even a 3-mmHg difference between brachial and central SBP was associated with improved cardiovascular outcomes. Further, several studies have shown that an increase in central SBP of only 1 mmHg has a substantial impact on large arterial remodeling [30] and silent cerebral damage [31]. Our findings therefore emphasize the importance of measuring serum FFA levels as a potential factor that modulates central hemodynamics, and of measuring central BP in epidemiological and clinical settings.

Several limitations to the present study warrant mention. First, we did not directly measure transit time and magnitude of reflection pressure wave. As transit time largely correlate with baPWV, we deduced from results of the regression analysis that reduced magnitude rather than delayed arrival of reflection pressure wave might be involved in the paradoxical relationship between FFA and better central hemodynamic profiles. More detailed waveform analysis would be needed to obtain conclusive evidence. Second, as this was a cross-sectional study, a longitudinal study is required to confirm the prognostic significance of central SBP differences arising from differences in serum FFA levels. Third, no information on the class of antihypertensive drugs was available for the Nagahama cohort sample, though beta-blockers and vasodilators have substantial class-effects on central BP

that are well documented [4-8]. Given that the associations of FFA quartile with AIx and PPa were independent of antihypertensive medication, our results might be non-differential and independent of the class-effects of antihypertensive drugs.

PERSPECTIVES

In conclusion, we found that serum FFA level is an important factor influencing central hemodynamics. Our results might help identify the as yet unidentified mechanisms behind the favorable effects of insulin resistance and type 2 diabetes on the central hemodynamic profile.

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CONFLICTS OF INTERESTS

The authors have no conflicts of interest to disclose.

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NOVELTY AND SIGNIFICANCE

What is new?

- Elevated serum free fatty acid levels were strongly associated with reduced magnitude of arterial reflection pressure wave and relatively low central blood pressure.
- Central pressure differs by approximately 4.9 mmHg due to serum FFA levels and diabetic status.

What is relevant?

Insulin resistance and diabetic status have been shown to be favorably associated with arterial waveform and central blood pressure. Serum free fatty acid was a more prominent determinant of arterial waveform than insulin.

Summary

Serum FFA was a factor that was favorably associated with central hemodynamics. A favorable association of FFA with central BP and arterial waveform suggests the importance of insulin signaling as a modulator of central hemodynamics.

FIGURE LEGENDS

Figure 1. Histogram of serum FFA level (study panel).

Serum FFA level was significantly higher in female participants than in males (ANOVA $P<0.001$).

Figure 2. Association of FFA quartile with central hemodynamic parameters (study panel).

Numbers of study participants in each subgroup are shown in the column. Statistical significance was assessed by analysis of variance. **A**, **B** and **C**, PP amplification; **D**, augmentation index; **E**, baPWV

Table 1. Clinical characteristics of study subjects

		Study panel (4,322)	Replication panel (5,071)
Age (years)		53±13	53±13
Sex (male %)		31.6	32.9
Body height (cm)		160.1±8.4	159.9±8.5
Body weight (kg)		57.0±10.9	57.3±10.9
Body mass index (kg/m^2)		22.1±3.2	22.4±3.3
Waist circumference (cm)*		79.8±9.8	80.4±9.3
Medication (%)	Hypertension	15.6	16.1
	Hyperglycemia	2.4	2.4
	Dyslipidemia	11.5	10.9
Brachial SBP (mmHg)		123±18	124±18
Central SBP (mmHg)		114±19	114±18
DBP (mmHg)		76±11	76±11
PP amplification (mmHg)		9±6	10±6
Radial AIx (%)		81.6±13.4	79.8±13.4
Heart rate (beats/min)		69±10	70±10
baPWV (cm/sec)		1,261±231	1,262±227
Type 2 diabetes		4.0	3.6
Glucose (mg/dl)		90±14	90±16
HbA1c (%)		5.5±0.5	5.4±0.5
Insulin (μU/ml)		5.0±3.1	5.7±6.1
Total cholesterol (mg/dl)		207±34	207±35
HDL cholesterol (mg/dl)		66±17	65±17
LDL cholesterol (mg/dl)		123±31	123±31
Triglyceride (mg/dl)		91±58	103±68
FFA (mEq/l)		0.69±0.24	0.78±0.31

Values are mean ± standard deviation. The study panel consisted of individuals whose fasting blood specimens (>11 h) were available, and the replication panel consisted of individuals whose peripheral blood samples were drawn within 10 h of their last meal. *Data available for 4,320 (study panel) and 5,069 (replication panel) subjects. Type 2 diabetes was defined as one or more of: fasting plasma glucose ≥126 mg/dl, occasional plasma glucose ≥200 mg/dl, HbA1c ≥6.5%, or taking oral antihyperglycemic drugs. AIx, augmentation index; baPWV, brachial-to-ankle pulse wave velocity; FFA, free fatty acid

Table 2. Differences in metabolic parameters among the quartile of PP amplification (study panel)

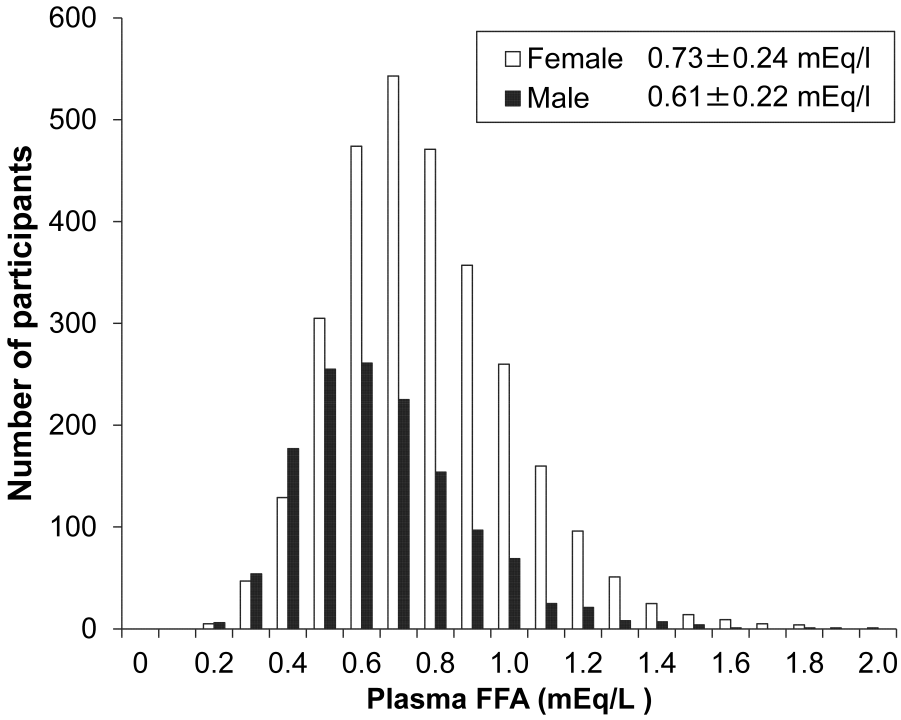
		Q1	Q2	Q3	Q4	*P*	
Range (mmHg)	Male	<6.5	6.5 to 9.9	10.0 to 14.4	>=14.5	Crude	Adjusted
	Female	<4.5	4.5 to 7.4	7.5 to 10.9	>=11.0		
No. of subjects		(982)	(1,143)	(1,101)	(1,096)		
Age (years old)		57±11	56±12	53±13	47±14	<0.001	
Body height (cm)		158.5±7.9	159.0±8.0	160.4±8.1	162.6±8.8	<0.001	
Body weight (kg)		55.6±9.7	56.7±10.2	57.0±10.6	58.7±12.4	<0.001	
Brachial SBP (mmHg)		125±19	122±17	122±18	123±19	0.006	
Heart rate (beats/min)		64±8	67±9	70±10	74±11	<0.001	
Glucose (mg/dl)		90±13	90±11	91±16	91±14	0.026	<0.001
Insulin (μU/ml)		4.6±2.8	5.0±2.9	5.0±2.9	5.5±3.5	<0.001	<0.001
HOMA-IR		1.04±0.70	1.13±0.74	1.16±0.77	1.26±1.00	<0.001	<0.001
HbA1c (%)		5.5±0.4	5.5±0.4	5.5±0.6	5.3±0.6	0.294	0.006
Total cholesterol (mg/dl)		210±33	208±33	207±34	202±36	<0.001	0.362
HDL cholesterol (mg/dl)		66±17	65±16	66±17	66±17	0.149	0.070
LDL cholesterol (mg/dl)		126±30	125±30	123±31	119±32	<0.001	0.022
Triglyceride (mg/dl)		91±51	93±57	90±53	90±69	0.618	0.337
FFA (mEq/l)		0.63±0.23	0.68±0.23	0.71±0.24	0.74±0.25	<0.001	<0.001

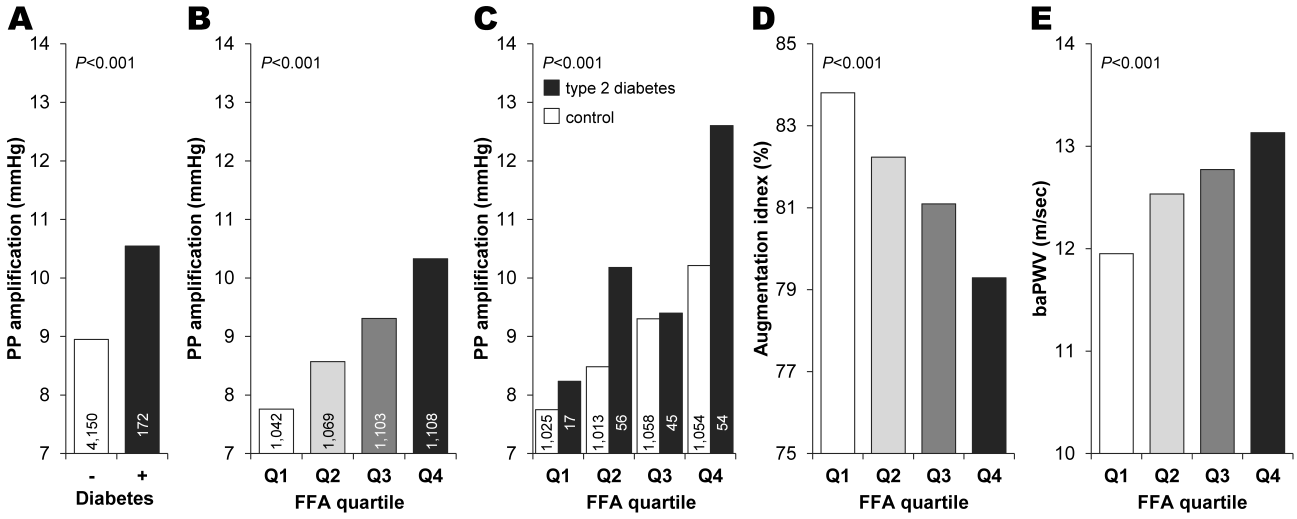
Values are mean ± standard deviation. Study subjects were divided into quartile by PP amplification within sex and then combined to avoid potential sex differences. Statistical significance was assessed by analysis of variance (crude model). *P*-values adjusted for age, sex, body height, body weight, and use of antihyperglycemic or lipid level-lowering drugs were obtained by linear regression analyses (adjusted model).
HOMA-IR, homeostasis model assessment of insulin resistance; FFA, free fatty acid

Table 3. Multiple linear regression analysis for central hemodynamic parameters

		PP amplification		AIx		Central SBP	baPWV
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Study panel (n=4,322)	Type 2 diabetes	0.027 (0.039)	0.015 (0.007)	-0.014 (0.233)	-0.012 (0.315)	-0.008 (0.039)	0.061 (<0.001)
	Insulin (log-transformed)	0.003 (0.855)	0.019 (0.004)	0.023 (0.100)	0.016 (0.241)	-0.001 (0.855)	0.071 (<0.001)
	AIx (%)		-1.004 (<0.001)				
	FFA (mEq/l)	0.146 (<0.001)	0.022 (<0.001)		-0.123 (<0.001)	-0.044 (<0.001)	0.069 (<0.001)
Replication panel (n=5,071)	Type 2 diabetes	0.038 (0.001)	0.020 (<0.001)	-0.016 (0.130)	-0.018 (0.082)	-0.012 (0.001)	0.055 (<0.001)
	Insulin (log-transformed)	0.028 (0.043)	0.037 (<0.001)	0.049 (<0.001)	0.009 (0.497)	-0.009 (0.043)	0.082 (<0.001)
	AIx (%)		-1.003 (<0.001)				
	FFA (mEq/l)	0.138 (<0.001)	0.023 (<0.001)		-0.115 (<0.001)	-0.045 (<0.001)	0.051 (<0.001)

Values are standardized regression coefficients (β). *P*-values are shown in parenthesis. Adjusted factors were as follows: age, sex, body height, body weight, taking medication for hypertension or dyslipidemia, SBP, heart rate, total cholesterol, and baPWV. In regression Model 6, heart rate and baPWV were not adjusted. AIx, augmentation index; FFA, free fatty acid; baPWV, brachial-to-ankle pulse wave velocity





SUPPLEMENTAL MATERIALS

Association of serum free fatty acid level with reduced reflection pressure wave magnitude and central blood pressure The Nagahama Study

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Table S1. Correlation coefficient for serum FFA levels (study panel)

	r	*P*
Age (years old)	0.087	<0.001
Body mass index (kg/m^2)	-0.084	<0.001
Brachial SBP (mmHg)	0.057	0.002
Central SBP (mmHg)	0.018	0.243
DBP (mmHg)	0.009	0.547
Glucose (mg/dl)	0.016	0.295
Insulin (μU/ml)	-0.023	0.125
HOMA-IR	-0.010	0.503
Total cholesterol (mg/dl)	0.085	<0.001
HDL cholesterol (mg/dl)	0.190	<0.001
LDL cholesterol (mg/dl)	-0.004	0.807
Triglyceride (mg/dl)	-0.034	0.027

HOMA-IR, homeostasis model assessment of insulin resistance.

Table S2. Differences in metabolic parameters among the FFA quartile (study panel)

		Q1	Q2	Q3	Q4	*P*	
Range (mEq/l)	Male	<0.45	0.45 to 0.57	0.58 to 0.72	>=0.73	Crude	Adjusted
	Female	<0.56	0.56 to 0.69	0.70 to 0.86	>=0.87		
No. of subjects		(1,042)	(1,069)	(1,103)	(1,108)		
Age (years old)		50±13	53±13	54±13	55±14	<0.001	
Body height (cm)		161.1±8.5	160.5±8.2	159.9±8.3	159.2±8.3	<0.001	
Body weight (kg)		57.4±10.0	57.8±11.2	57.1±10.9	55.8±11.2	<0.001	
Heart rate (beats/min)		66±9	68±10	70±10	72±11	<0.001	
Glucose (mg/dl)		88±10	91±12	91±14	92±16	<0.001	<0.001
Insulin (μU/ml)		5.0±3.0	5.1±3.0	5.2±3.2	4.9±3.0	0.306	0.053
HOMA-IR		1.10±0.74	1.16±0.79	1.19±0.84	1.15±0.88	0.081	0.004
HbA1c (%)		5.4±0.4	5.5±0.4	5.5±0.5	5.5±0.7	<0.001	0.005
Total cholesterol (mg/dl)		203±33	206±33	208±34	211±35	<0.001	<0.001
HDL cholesterol (mg/dl)		64±16	64±17	66±17	69±17	<0.001	<0.001
LDL cholesterol (mg/dl)		122±30	124±31	123±30	124±32	0.437	0.866
Triglyceride (mg/dl)		86±47	92±54	93±56	92±72	0.022	0.030

Values are mean ± standard deviation. Study subjects were divided into FFA quartile within sex and then combined to avoid potential sex differences. Statistical significance was assessed by analysis of variance (crude model). *P*-values adjusted for age, sex, body height, body weight, and use of antihyperglycemic or lipid level-lowering drugs were obtained by linear regression analyses (adjusted model). HOMA-IR, homeostasis model assessment of insulin resistance; FFA, free fatty acid

Table S3. Multiple linear regression analysis for central hemodynamic parameters by sex

		PP amplification		AIx		cSBP	baPWV
		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Male (n=3,031)	Type 2 diabetes	0.034 (0.027)	0.027 (<0.001)	-0.006 (0.662)	-0.006 (0.643)	-0.013 (0.026)	0.077 (<0.001)
	Insulin (log-transformed)	0.060 (0.001)	0.045 (<0.001)	0.033 (0.038)	-0.014 (0.386)	-0.023 (0.001)	0.093 (<0.001)
	AIx (%)		-1.035 (<0.001)				
	FFA (mEq/l)	0.164 (<0.001)	0.026 (<0.001)		-0.134 (<0.001)	-0.063 (<0.001)	0.087 (<0.001)
Female (n=6,362)	Type 2 diabetes	0.039 (<0.001)	0.010 (0.039)	-0.027 (0.006)	-0.029 (0.003)	-0.010 (<0.001)	0.044 (<0.001)
	Insulin (log-transformed)	0.006 (0.629)	0.024 (<0.001)	0.047 (<0.001)	0.018 (0.117)	-0.002 (0.629)	0.080 (<0.001)
	AIx (%)		-1.013 (<0.001)				
	FFA (mEq/l)	0.158 (<0.001)	0.031 (<0.001)		-0.125 (<0.001)	-0.042 (<0.001)	0.040 (<0.001)

Values are standardized regression coefficient (β). *P*-values are shown in parenthesis. Adjusted factors were as follows; age, body height, body weight, taking medication for hypertension or dyslipidemia, SBP, heart rate, total cholesterol and baPWV. In the regression model 6, heart rate and baPWV were not adjusted. AIx, augmentation index; FFA, free fatty acid; baPWV, brachial-to-ankle pulse wave velocity.

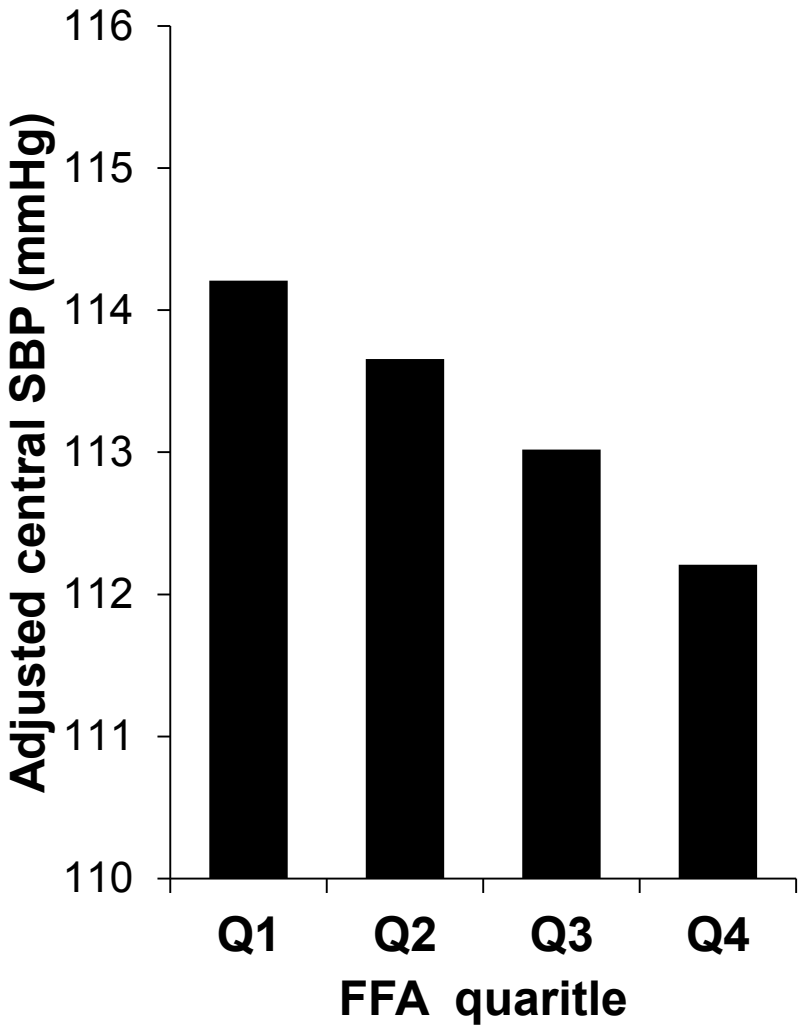

Figure S1 Mean adjusted central SBP levels by FFA quartile (Study panel)

Adjusted factors were as follows: age, sex, body height, body weight, taking medication for hypertension or dyslipidemia, SBP, heart rate, total cholesterol, insulin, baPWV, and type 2 diabetes. Overall *P*-value was <0.001.